

Appln. No. 09/935,338
Amd. dated January 23, 2004
Reply to Office Action of October 27, 2003

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-220 (Cancelled).

221 (Currently amended). A method for amplifying a nucleic acid, characterized in that the method comprises:

(a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; and

(b) incubating the reaction mixture for a sufficient time to generate a reaction product under conditions where specific annealing of the primer to the nucleic acid as the template, an extended strand synthesis reaction and a strand

displacement reaction by the DNA polymerase, and a reaction of cleaving the extended strand by the endonuclease take place.

222(Previously presented). The method according to claim 221, wherein the reaction mixture is incubated isothermally.

223(Currently amended). The method according to claim 221, wherein the reaction mixture further contains [[a]] another chimeric oligonucleotide primer having a sequence substantially homologous to the nucleotide sequence of the nucleic acid as the template.

224(Previously presented). The method according to claim 221, wherein the DNA polymerase is selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax.

225(Previously presented). The method according to claims 221, wherein the endonuclease is an endoribonuclease.

226(Previously presented). The method according to claim 225, wherein the endoribonuclease is RNase H.

227(Previously presented). The method according to claim 226, wherein the RNase H is selected from the group

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consisting of an RNase H from *Escherichia coli*, an RNase H from a bacterium of genus *Thermotoga*, an RNase H from a bacterium of genus *Thermus*, an RNase H from a bacterium of genus *Pyrococcus*, an RNase H from a bacterium of genus *Archaeoglobus* and an RNase H from a bacterium of genus *Bacillus*.

228(Previously presented). The method according to claim 221, wherein the DNA polymerase having a strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax* and the RNase H as an endonuclease is selected from the group consisting of an RNase H from *Escherichia coli*, an RNase H from a bacterium of genus *Pyrococcus* and an RNase H from a bacterium of genus *Archaeoglobus*.

229(Previously presented). The method according to claim 228, wherein the RNase H is type I RNase H from *Escherichia coli*, or type II RNase H from a bacterium of genus *Pyrococcus* or a bacterium of genus *Archaeoglobus*.

230(Previously presented). The method according to claim 221, wherein a DNA polymerase having an endonuclease activity is used.

231(Previously presented). The method according to claim 230, wherein the DNA polymerase is Bca DNA polymerase

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lacking 5'→3' exonuclease from *Bacillus caldotenax* and the Bca DNA polymerase is used in the presence of a substance that allows the endonuclease activity of the Bca DNA polymerase to express.

232(Previously presented). The method according to claim 231, wherein the substance that allows the endonuclease activity of the DNA polymerase to express is a manganese ion.

233(Previously presented). The method according to claim 221, wherein the amplification reaction is conducted in the presence of a substance that inhibits the reverse transcription activity of the DNA polymerase.

234(Previously presented). The method according to claim 233, wherein the substance that inhibits the reverse transcription activity of the DNA polymerase is phosphonoformic acid.

235(Previously presented). The method according to claim 221, wherein the chimeric oligonucleotide primer contains two or more successive ribonucleotide residues.

236(Previously presented). The method according to claim 221, wherein the chimeric oligonucleotide primer contains one or more modified ribonucleotide.

237(Previously presented). The method according to claim 236, wherein the chimeric oligonucleotide primer contains an (α -S) ribonucleotide in which the oxygen atom bound to the phosphorous atom at the α -position of the ribonucleotide is replaced by a sulfur atom.

238(Currently amended). The method according to claim 221, wherein a chimeric oligonucleotide primer represented by general formula below is used:

General formula: 5'-dNa-Nb-dNc-3'

wherein a is an integer of 11 or more; b is an integer of 1 or more; c is 0 or an integer of 1 or more; dN is an deoxyribonucleotide and/or nucleotide analog; N is an unmodified ribonucleotide and/or modified ribonucleotide, wherein some of dNs in dNa may be replaced by Ns, and the nucleotide at the 3'-terminus may be modified such that extension from the 3'-terminus by the action of the DNA polymerase does not take place (~~a: an integer of 11 or more; b: an integer of 1 or more; c: 0 or an integer of 1 or more; dN: deoxyribonucleotide and/or nucleotide analog; N: unmodified ribonucleotide and/or modified ribonucleotide, wherein some of dNs in dNa may be replaced by Ns, and the nucleotide at the 3'-terminus may be modified such that extension from the 3'-terminus by the action of the DNA polymerase does not take place~~).

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239(Previously presented). The method according to claim 238, wherein c is 0.

240(Previously presented). The method according to claim 238, wherein the nucleotide analog is deoxyriboinosine nucleotide or deoxyribouracil nucleotide, and the modified ribonucleotide is (α -S) ribonucleotide.

241(Previously presented). The method according to claim 238, wherein the nucleic acid amplification reaction is conducted at a temperature suitable for the chimeric oligonucleotide primer as defined in claim 238.

242(Currently amended). The method according to claim 221, wherein the chimeric oligonucleotide primer is at least one chimeric oligonucleotide primer selected from the group consisting of:

a) a chimeric oligonucleotide primer for detecting enterohemorrhagic Escherichia coli having a nucleotide sequence selected from the group consisting of SEQ ID NOS: ~~31 to 34, 47, 48, 51-53, 64-72, 84, 85, 113, 114, 130 and 131~~ 43 to 46, 136, 137, 140-142, 153-161, 173, 174, 202, 203, 219 and 220;

b) a chimeric oligonucleotide primer for detecting a viroid having a nucleotide sequence selected from the group consisting of SEQ ID NOS: ~~59, 60, 119, 120, 122 and 123~~ 148, 149, 208, 209, 211, 212;

c) a chimeric oligonucleotide primer for detecting *Clostridium botulinum* having a nucleotide sequence represented by SEQ ID NO: ~~116~~ 205 or ~~117~~ 206;

d) a chimeric oligonucleotide primer for detecting papilloma virus having a nucleotide sequence represented by SEQ ID NO: ~~96~~ 185 or ~~97~~ 186;

e) a chimeric oligonucleotide primer for detecting hepatitis C virus having a nucleotide sequence selected from the group consisting of SEQ ID NOS: ~~101, 102, 138, 139, 200, 201, 205 and 206~~ 190, 191, 227, 228, 289 and 290;

f) a chimeric oligonucleotide primer for detecting *Staphylococcus aureus* having a nucleotide sequence represented by SEQ ID NO: ~~136~~ 225 or ~~137~~ 226;

g) a chimeric oligonucleotide primer for detecting *Mycobacterium tuberculosis* having a nucleotide sequence selected from the group consisting of SEQ ID NOS: ~~155, 156, 159 to 162, 194 and 195~~ 244, 245, 248 to 251, 283, 284; and

h) a chimeric oligonucleotide primer for detecting *Chlamydia* having a nucleotide sequence selected from the group consisting of SEQ ID NOS: ~~157, 158, 203 and 204~~ 246 and 247.

243 (Previously presented). The method according to claim 221, which is conducted in a buffer that contains a buffering component selected from the group consisting of Tricine, a phosphate, tris, Bicine and HEPES.

244(Previously presented). The method according claim 221, wherein the nucleic acid as the template is a single-stranded DNA or a double-stranded DNA.

245(Previously presented). The method according to claim 244, which is conducted after converting a double-stranded DNA as the template into single-stranded DNAs.

246(Previously presented). The method according to claim 244, wherein the nucleic acid as the template is a cDNA obtained from an RNA by a reverse transcription reaction.

247(Previously presented). The method according to claim 246, which is conducted after synthesizing a cDNA by a reverse transcription reaction using an RNA as a template.

248(Previously presented). The method according to claim 247, wherein a primer selected from the group consisting of an oligo-dT primer, a random primer and a specific primer is used as a primer for the reverse transcription reaction.

249(Previously presented). The method according to claim 247, wherein a chimeric oligonucleotide primer is used as a primer for the reverse transcription reaction.

250(Previously presented). The method according to claim 247, wherein a DNA polymerase having a reverse transcriptase activity is used as a reverse transcriptase.

251(Previously presented). The method according to claim 247, wherein the reverse transcription reaction and the nucleic acid amplification reaction are conducted using one DNA polymerase having a reverse transcriptase activity and a strand displacement activity.

252(Previously presented). The method according to claim 251, wherein the DNA polymerase is Bst DNA polymerase lacking 5'→3' exonuclease from *Bacillus stearothermophilus* or Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax*.

253(Previously presented). The method according to claim 246, wherein the RNA as the template in the reverse transcription reaction is an RNA amplified by an additional nucleic acid amplification reaction.

254(Previously presented). The method according to claim 253, which is conducted after synthesizing an amplified RNA fragment by an additional nucleic acid amplification reaction using an RNA as a template.

255(Previously presented). The method according to claim 253, wherein the additional nucleic acid amplification reaction is selected from the group consisting of the transcription-based amplification system (TAS) method, the self-sustained sequence replication (3SR) method, the nucleic acid sequence-based amplification (NASBA) method, the transcription-mediated amplification (TMA) method and the Q β replicase method.

256(Previously presented). The method according to claim 244, wherein the nucleic acid as the template is a DNA obtained by an additional nucleic acid amplification reaction.

257(Previously presented). The method according to claim 256, which is conducted after synthesizing an amplified DNA fragment by an additional nucleic acid amplification reaction using an DNA as a template.

258(Previously presented). The method according to claim 256, wherein the additional nucleic acid amplification reaction is selected from the group consisting of the polymerase chain reaction (PCR) method, the ligase chain reaction (LCR) method and the strand displacement amplification (SDA) method.

259(Previously presented). The method according to claim 253, wherein a random primer or a degenerate primer is used for the additional nucleic acid amplification reaction.

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260 (Previously presented). The method according to claim 259, wherein the random primer or the degenerate primer is a primer having a random sequence or a degenerate sequence at least at the 3'-terminus or on the 3'-terminal side.

261 (Previously presented). The method according to claim 256, wherein a random primer or a degenerate primer is used for the additional nucleic acid amplification reaction.

262 (Previously presented). The method according to claim 261, wherein the random primer or the degenerate primer is a primer having a random sequence or a degenerate sequence at least at the 3'-terminus or on the 3'-terminal side.

263 (Previously presented). The method according to claim 221, wherein the length of the region of the nucleic acid to be amplified is 200 bp or shorter.

264 (Previously presented). The method according to claim 221, which comprises annealing the nucleic acid as the template to the chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid in an annealing solution containing a substance that enhances the annealing of the nucleic acid to the primer.

265(Previously presented). The method according to claim 264, wherein the annealing solution contains spermidine and/or propylenediamine.

266(Previously presented). The method according to claim 264, wherein the annealing is conducted by incubating the annealing solution containing the nucleic acid as the template and the chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid at 90°C or above and then cooling the solution to a temperature at which the amplification reaction is conducted or below.

267(Previously presented). The method according to claim 221, wherein the nucleic acid amplification reaction is conducted in the presence of a deoxyribonucleotide triphosphate analog.

268(Previously presented). The method according to claim 267, wherein the deoxyribonucleotide triphosphate analog is deoxyuridine triphosphate or a derivative thereof.

269(Previously presented). The method according to claim 221, wherein a template switching reaction is effected.

270(Previously presented). The method according to claim 221, which further comprises a step of duplicating a DNA or an RNA containing a sequence to be amplified to prepare a nucleic

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acid as a template prior to step (a), wherein the duplicated nucleic acid is used in step (a) as a nucleic acid as a template.

271(Previously presented). A method for producing a nucleic acid in large quantities, characterized in that the method comprises:

(a) amplifying a nucleic acid by the method defined by claim 221; and

(b) collecting the nucleic acid amplified in step (a).

272(Previously presented). A method for detecting a target nucleic acid in a sample, characterized in that the method comprises:

(a) amplifying a target nucleic acid by the method defined by claim 221; and

(b) detecting the target nucleic acid amplified in step (a).

273(Previously presented). The method according to claim 272, which comprises detecting the amplified nucleic acid using a probe for detection.

274(Previously presented). The method according to claim 273, wherein the probe for detection is a probe that has been labeled with a labeling substance.

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275(Previously presented). The method according to claim 274, wherein the probe is an RNA probe labeled with two or more fluorescent substances positioned at a distance that results in a quenching state.

276(Currently amended). The method according to claim 273, wherein the probe is a probe which hybridizes to a region amplified using at least one chimeric oligonucleotide primer selected from the group consisting of:

a) a chimeric oligonucleotide primer for detecting enterohemorrhagic Escherichia coli having a nucleotide sequence selected from the group consisting of SEQ ID NOS: ~~31 to 34, 47, 48, 51-53, 64-72, 84, 85, 113, 114, 130 and 131~~ 43 to 46, 136, 137, 140-142, 153-161, 173, 174, 202, 203, 219 and 220;

b) a chimeric oligonucleotide primer for detecting a viroid having a nucleotide sequence selected from the group consisting of SEQ ID NOS: ~~59, 60, 119, 120, 122 and 123~~ 148, 149, 208, 209, 211 and 212;

c) a chimeric oligonucleotide primer for detecting Clostridium botulinum having a nucleotide sequence represented by SEQ ID NO: ~~116~~ 205 or ~~117~~ 206;

d) a chimeric oligonucleotide primer for detecting papilloma virus having a nucleotide sequence represented by SEQ ID NO: ~~96~~ 185 or ~~97~~ 186;

e) a chimeric oligonucleotide primer for detecting hepatitis C virus having a nucleotide sequence selected from the group consisting of SEQ ID NOS: ~~10221, 102, 138, 139, 200, 201, 205 and 206~~ 190, 191, 227, 228, 289 and 290;

f) a chimeric oligonucleotide primer for detecting Staphylococcus aureus having a nucleotide sequence represented by SEQ ID NO: ~~136~~ 225 or ~~137~~ 226;

g) a chimeric oligonucleotide primer for detecting Mycobacterium tuberculosis having a nucleotide sequence selected from the group consisting of SEQ ID NOS: ~~155, 156, 159 to 162, 194 and 195~~ 244, 245, 248 to 251, 283, 284; and

h) a chimeric oligonucleotide primer for detecting Chlamydia having a nucleotide sequence selected from the group consisting of SEQ ID NOS: ~~157, 158, 203 and 204~~ 246 or 247.

277(Previously presented). A method for determining a nucleotide sequence of a nucleic acid, characterized in that the method comprises:

(a) amplifying a nucleic acid by the method defined by claim 221; and

(b) determining the nucleotide sequence of the nucleic acid amplified in step (a).

278(Previously presented). A method for preparing a single-stranded nucleic acid, the method comprising generating a

single-stranded nucleic acid using the method defined by claim 221.

279(Previously presented). The method according to claim 278, wherein at least two primers at different concentrations are used.

Claims 280-304 (Cancelled).

305(Previously presented). A method for amplifying a nucleotide sequence, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with an endonuclease at a site that contains the ribonucleotide; and

(c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-

extended strand is cleaved obtained in step (b) to effect a strand displacement.

306(Previously presented). A method for amplifying a nucleotide sequence using at least two primers, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with an endonuclease at a site that contains the ribonucleotide;

(c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (b);

(d) treating a released displaced strand obtained in step (c) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(e) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (d) with an endonuclease at a site that contains the ribonucleotide; and

(f) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (e).

Claims 307 and 308 (Cancelled).

309(Previously presented). A method for amplifying a nucleic acid, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template and synthesize a double-stranded nucleic acid, wherein the primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid; and

(c) reusing in step (b) the double-stranded nucleic acid obtained in step (b) as a template.

310(Previously presented). The method according to claim 309, wherein the DNA polymerase is at least one DNA polymerase having a strand displacement activity.

311 (Currently amended). A method for amplifying a nucleic acid using at least two primers, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid;

(c) reusing in step (b) the double-stranded nucleic acid obtained in step (b) as a template;

(d) treating a displaced strand obtained in step (b) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand,

wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a ribonucleotide as well as at least one selected from the group ~~consisting~~ consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(e) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid; and

(f) reusing in step (e) the double-stranded nucleic acid obtained in step (e) as a template.

312(Previously presented). The method according to claim 311, wherein the DNA polymerase is at least one DNA polymerase having a strand displacement activity.

313(Currently amended). A method for amplifying a nucleic acid, characterized in that the method comprises:

(a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-

stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group ~~consisting~~ consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; and

(c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the template and the primer-extended strand.

314(Currently amended). A method for amplifying a nucleic acid, characterized in that the method comprises:

(a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group ~~consisting~~ consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; and

(c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other.

315 (Currently amended). A method for amplifying a nucleic acid, characterized in that the method comprises:

(a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group ~~consisting~~ consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease;

(c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid

consisting of the primer-extended strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed;

(d) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid to which the two primers are annealed obtained in step (c) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed; and

(e) reusing in step (d) the double-stranded nucleic acid to which the two primers are annealed obtained in step (d).

316(Currently amended). A method for amplifying a nucleic acid, characterized in that the method comprises:

(a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended

strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group ~~consisting~~ consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease;

(c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed;

(d) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid to which the two primers are annealed obtained in step (c) to effect strand displacements and

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obtain a double-stranded nucleic acid consisting of the template and the primer-extended strand;

(e) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the template and the primer-extended strand obtained in step (d) with the endonuclease; and

(f) extending a nucleic acid that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to synthesize a displaced strand.

Claims 317-331 (Cancelled).

332(New). The method according to claim 305, wherein step (b) and step (c) are sequentially repeated.

333(New). The method according to claim 305, wherein the DNA polymerase in step (a) is different from the DNA polymerase having a strand displacement activity in step (c).

334(New). The method according to claim 306, wherein one DNA polymerase having a strand displacement activity is used.